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ORIGINAL ARTICLE

Low Prevalence of Antibodies to GAD65 in a 50- to 74-Year-Old General Dutch Population

The Hoorn Study

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OBJECTIVE — To assess the prevalence of antibodies to GAD65 (GAD65-A) in relation to glucose tolerance disturbances and to blood glucose-lowering therapy in a general Dutch population.

RESEARCH DESIGN AND METHODS — A population sample of 2,350 Dutch subjects, age 50–74 years, agreed to undergo an oral glucose tolerance test (OGTT). They were classified as having normal glucose tolerance, impaired glucose tolerance, newly detected diabetes, or known diabetes. GAD65-A levels were measured in serum by means of a standardized radioligand assay and subsequently were expressed as indexes. The prevalence rates were defined as the proportions of individuals of each category of glucose tolerance exceeding the value of the index at the 99th percentile of the entire study population.

RESULTS — The prevalence rates and the 95% CIs of GAD65-A were 0.7% (0.4–1.2%) in cases of normal glucose tolerance, 2.4% (0.9–5.3%) in impaired glucose tolerance, 0% (0–3.3%) in newly detected diabetes, according to the World Health Oganization (WHO) criteria, and 3.5% (0.7–10.0%) in known diabetes. A total of 2 out of 3 subjects with GAD65-A indexes above the 99th percentile and 10 out of 18 subjects with GAD65-A indexes above the 85th percentile received insulin therapy for their diabetes, which showed an association between GAD65-A and insulin therapy.

CONCLUSIONS — Low prevalence rates of latent autoimmunity to GAD were found in 50- to 74-year-old Dutch subjects with normal and abnormal glucose tolerance, and GAD65-A was associated with insulin use in known diabetic subjects.

Diabetes in adults is a heterogeneous disorder, with variation in clinical presentation. The majority of these patients present with characteristic features associated with insulin resistance and are classified as having NIDDM. A minority of patients present with features that are characteristic for neither NIDDM nor IDDM (1). Some have autoantibodies directed against the β-cell proteins of the pancreas, suggesting an autoimmune nature. In these patients, antibodies to GAD (GAD65-A) are specifically associated with difficulties in blood glucose regulation, which result in insulin therapy varying from a few weeks to a few years after clinical diagnosis (2,3). These patients were considered to have latent autoimmune diabetes (1,4). To establish the validity of GAD65-A in the prediction of the clinical course of adult-onset diabetes, a cross-sectional study was performed in which we measured GAD65-A in relation to glucose tolerance disturbances and to blood glucose–lowering diabetes therapy.

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CPM, counts per minute; GAD65-A, antibodies to the 65kD isoform of glutamic acid decarboxylase; OGTT, oral glucose tolerance test; WHO, World Health Organization.

RESEARCH DESIGN AND METHODS

Subjects

A random sample of 50- to 74-year-old subjects was taken from the population register of the town of Hoorn in the Netherlands (57,000 inhabitants). Of the 3,553 subjects invited, 2,540 (71.5%) participated, of whom 56 non-Caucasian subjects were excluded. Caucasian ethnicity was defined as having at least three grandparents from European or Mediterranean countries. The actual cohort therefore consisted of 2,484 subjects (5).

GAD65-A

Antibodies to the 65kD isoform of GAD were measured in serum and stored at −70°C by means of a radioligand assay as previously described (6,7). GAD65-A levels were expressed as index values measured in counts per minute (cpm). GAD65-A index = 1 + (cpm [unknown sample] − cpm [negative standard serum]) / (cpm [positive standard serum] − cpm [negative standard serum]). A constant of 1 was added to each index to facilitate interpretation, but this had no effect on the statistical tests. GAD65-A indexes showed positively skewed distributions as well as a minimum value for the general population of 0.9 and a maximum value of 5.8. GAD65-A indexes above the 99th percentile were arbitrarily defined as positive (6). Samples were tested three times and the mean value of these tests was used in the analysis. The interassay coefficient of variation was 7.5% (n = 72).

Oral glucose tolerance tests (OGTTs)

All subjects who were not treated with sulfonylurea, metformin, or insulin underwent a 75-g oral glucose tolerance test and were

Table 1—*GAD65-A indexes according to glucose tolerance category in a general Dutch population sample*

GAD65-A levels	Normal glucose tolerance	Impaired glucose tolerance	Newly detected diabetes	Known diabetes
n	1,909	245	111	85
>99.25th percentile	10 (0.5)	4 (1.6)	—	3 (3.5)†
>99th percentile*	14 (0.7)	6 (2.4)†	—	3 (3.5)†
>95th percentile	90 (4.7)	12 (4.9)	5 (4.5)	12 (14.1)†
>85th percentile	285 (14.9)	37 (15.1)	13 (11.7)	20 (23.5)†
Median (interquartile range)	0.977 (0.968–0.987)	0.976 (0.965–0.987)	0.976 (0.976–0.985)	0.981 (0.970–0.992)†

Data are *n* (% of corresponding category) or medians (interquartile range). GAD65-A levels are expressed as index values. *Approximate mean + 2 SD, GAD65-A indices above this value are defined as positive; †$P < 0.05$ vs. normal glucose tolerance.

classified according to the World Health Organization (WHO) (8) criteria as having normal glucose tolerance, impaired glucose tolerance, or newly detected diabetes. Verified known diabetes was defined as previously diagnosed diabetes treated with sulfonylurea, metformin, insulin, or diet-only, if the results of an OGTT met the WHO criteria for diabetes. The venous plasma glucose values were determined according to a glucose dehydrogenase method (Merck, Darmstadt, Germany). In 118 (4.8%) subjects, the GAD65-A could not be measured because of lack of serum, and in 16 subjects a glucose tolerance category could not be established because of an incomplete OGTT, resulting in 2,350 subjects being included in the analysis.

Biometry

BMI was calculated as weight (kilograms) divided by height (meters) squared.

Analysis

The differences in continuous variables between two groups were analyzed by means of the Student's *t* test for unpaired samples or the Mann-Whitney *U* test, when appropriate. A χ^2 test or Fisher's exact test was used to analyze differences between the groups in frequency data. All data were analyzed with an SPSS-PC software package, version 5.0 (SPSS, Chicago, IL), or Epitable (Coulombier, Charenton, France).

RESULTS — The prevalence rates and 95% CIs of antibodies to GAD65 were 0.7% (0.4–1.2%) in cases of normal glucose tolerance, 2.4% (0.9–5.3%) in impaired glucose tolerance, 0% (0–3.3%) in newly detected diabetes, and 3.5% (0.7–10.0%) in known diabetes. In each glucose tolerance category, GAD65-A indexes showed distributions that were positively skewed (distributions not shown). These distributions were similar for normal glucose tolerance, impaired glucose tolerance, and newly detected diabetes (Table 1). The distribution of GAD65-A indexes in known diabetes was significantly different from that in normal glucose tolerance. This difference is likely due to the GAD65-A indexes of subjects with known diabetes who were on insulin therapy: 10 out of 18 had GAD65-A indexes above the 85th percentile (Table 2). In addition, 3 subjects with known diabetes had GAD65-A indexes above the 99th percentile and were thus considered to be positive. Two of these three subjects, aged 50 and 54 years, were on insulin therapy (ages at onset of diabetes were 35 and 49 years). The third was 68 years old and was only on a diet (age at onset was 44 years). The mean BMI of the 23 subjects in the entire study population who had GAD65-A was 7.5% below the mean BMI of the remaining subjects without GAD65-A (Table 3). The mean age did not differ, and 70% of those with GAD65-A were female.

CONCLUSIONS — We found a marginal increase in prevalence rates of GAD65-A in known diabetes and impaired glucose tolerance compared with normal glucose tolerance and no increase in prevalence rates in newly detected diabetes. The increase in known diabetes was less evident than previously suggested (1,4,9). The skewness of the distributions of GAD65-A indexes convinced us to use percentiles rather than standard deviations to define

Table 2—*Characteristics of subjects with known diabetes according to blood glucose–lowering therapy*

	Diet only	Sulfonylurea and/or metformin	Insulin
n	12	55	18
GAD65-A index			
>99.25th percentile	1 (8.3)	—	2 (11.1)
>99th percentile*	1 (8.3)	—	2 (11.1)
>95th percentile	2 (16.7)	4 (7.3)†	6 (33.3)
>85th percentile	2 (16.7)	8 (14.5)†	10 (55.6)
Other characteristics			
Sex (F/M)	8/4	32/23	10/8
Age (years)	65 ± 4.7	66.2 ± 6.5	62.7 ± 6.5
BMI (kg/m^2)	24.8 ± 8.4	29.3 ± 5.2	27.4 ± 4.2
Fasting plasma glucose (mmol/l)	9.6 ± 2.9†	10.3 ± 3.5†	12.7 ± 3.7
Duration of diabetes (years)	11.1 ± 19.2	9.7 ± 12.4	15.4 ± 16.2
Onset of diabetes (years)	54.5 ± 19.6	56.6 ± 14.5†	47.3 ± 15.1

Data are means ± SD or *n* (% of corresponding category). *Approximate mean + 2 SD, GAD65-A indexes above this value are defined as positive; †$P < 0.05$ vs. insulin therapy.

Table 3—*Characteristics of the study population in relation to the presence or absence of antibodies to GAD65-A*

	GAD65-A	
	Presence†	Absence
n	23	2,327
Sex (F/M)	16/7	1,236/1,091
Age (years)	62.2 ± 7.8	61.7 ± 7.4
BMI (kg/m^2)	24.5 ± 6.4*	26.5 ± 3.5

Data are *n* or means ± SD. *$P = 0.008$ vs. absence of GAD65-A; †GAD65-A indexes above the 99th percentile.

positivity, using a threshold for GAD65-A at the 99th percentile (index value 1.2). This almost coincides with the mean of the population + 2 SD (index value 1.4). A recent report with follow-up data on GAD65-A–positive diabetes patients suggested that only high levels of GAD-antibodies predict the need for insulin treatment (10). We considered several thresholds and tested all samples again with an index in the high range of the radioligand assay by means of a classical immunoprecipitation assay, evaluated by SDS-PAGE (11). The present data retrieved from a general population sample showed an overall prevalence rate of GAD65-A, similar to that found in an earlier Dutch general population study (12), and a rate of 3.5% for the category of known diabetes. In this category, the association between high GAD65-A indexes and insulin therapy suggests ongoing β-cell destruction. However, the prevalence rate is low compared to the findings of other studies in adult-onset diabetes. These studies have reported varying rates, possibly because of ethnic differences or the use of different criteria for the selection of study subjects. Prevalence rates were reported to be 4.3% in Japan (13), 1.7% in Korea (14), and 1.1% in Papua New Guinea (15), while for white subjects, adult rates between 3 and 36% have been reported (9,16,17). Other explanations for these discrepancies could be differences in applied assays for GAD antibodies and threshold selection. It is also possible that IDDM subjects in the Netherlands develop the disease at a younger age, compared with a population with a higher prevalence rate of GAD65A. A high proportion of insulin-requiring diabetic subjects showed GAD65-A indexes above the 85th percentile (Table 2). The most likely explanation for that intriguing phenomenon is that these diabetic subjects once were positive (GAD65A indexes above the 99th percentile) and that the duration of the disease resulted in a decreased level. No explanation has been found for the association between a low BMI and GAD65-A positivity in the population. We used GAD antibodies instead of other diabetes-specific antibody markers, since it seems to be the most sensitive marker in older persons (18). We conclude that the prevalence of GAD65-A in Dutch Caucasian subjects is low in any category of glucose tolerance and that distributions of GAD65-A indexes were similar for normal glucose tolerance, impaired glucose tolerance, and newly detected diabetes. Although this cross-sectional study does not allow definite conclusions, an important role for GAD65-A as a predictive marker for insulin dependency in Dutch subjects who present with diabetes after the age of 50 appears to be very unlikely. The results also suggest a low frequency of latent autoimmunity in such a population.

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